Evaluation of Chemicals for Toxic & Teratogenic Effects Using the Chick Embryo as the Test System-FDA Contract 71-331 (**Sodium Nitrite**) No Date

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FDA CONTRACT 71-331

EVALUATION OF CHEMICALS FOR TOXIC AND TERATOGENIC EFFECTS USING THE CHICK EMBRYO AS THE TEST SYSTEM

Sodium Nitrite

Objective: To determine the toxic and teratogenic effects of GRAS List compounds when injected into the air cell and yolk of fertile chicken eggs.

Procedure:

A. Test System and Incubation Procedures:

Fertile hatching eggs were chosen from a single comb white leghorn breeder flock. The eggs were candled and graded to eliminate internal and external defects; blood and meat spots, tremulous air cells, rough or cracked shells. The eggs chosen for injection weighed from 23 - 26 ounces/dozen and were not washed or dipped. The eggs were gathered within 48 hours of the injection or incubation and were held at 50 - 60°F. and 60 - 80% relative humidity. The breeder ration fed the flock was formulated by the breeder to meet or exceed the recommendations of the Nutrient Requirements of Poultry, Number 1 - 1971, National Academy of Sciences, and contained no additions of antibiotics, arsenicals, nitrofurazones or similar chemical additives. The breeder flock was blood tested and negative for pullorum-typhoid and mycoplasm gallispecticum.

Eggs were incubated in Jamesway 1080 forced air incubators equipped with automatic controls to regulate temperature, humidity and egg turning. Temperature and relative humidity were maintained at 99.5°F. and 86°F.* wet bulb respectively for the first 18 days of incubation and eggs were turned each two hours. The eggs were then transfered to the hatcher in 3½" x 5" x 25" covered hardware cloth hatching baskets for the hatching period. Temperature in

* 86°F. wet bulb refers to temperature of wet bulb apparatus in standard incubator hatching equipment and is equivalent to approximately 56% relative humidity.

the hatcher was maintained at 98.5oF. and relative humidity at 86oF. wet bulb. When the humidity had risen to 88 degrees as a result of moisture generated by hatching the hatcher was adjusted to hold 88oF. wet bulb relative humidity until the chicks were removed on the morning of the 23rd day of incubation.

Prior to incubating or hatching each setting of eggs and following each hatching the incubator and its metal parts were thoroughly cleaned by vacuuming and washing with a 200 ppm solution of "ROCCAL" which contains 10% alkyl (C12, C14, C16 and related alkyl groups from C8 to C18) - dimethyl benzyl ammonium chloride. The sanitized surfaces were allowed to completely air dry prior to the introduction of eggs. Following each transfer of eggs to the hatching compartment, and when temperature and humidity had returned to normal levels, the hatcher and the eggs it contained were fumigated by combining 10 grams of potassium permanganate crystals and 20 grams of 37% formaldahyde solution.

B. Test Sample Preparation and Administration:

The test sample was taken up in an appropriate solvent to facilitate administration at the levels chosen. Sterile glassware, syringes and needles were employed to prepare and administer the test sample or solvent. Eggs previously selected were candled and the location of the air cell marked with pencil. The eggs were then randomized into the experimental groups. Injection of solvent or test sample dilution was accomplished by placing the material on the air cell membrane or by injection into the yolk sac. These administrations were made at both 0 and 96 hours of incubation. For the 0 hour experiments the eggs were assumed to be fertile; however, in the 96 hour experiments the eggs were candled as previously described and only those eggs with a well developed 96 hour embryo were selected for use.

1. Air Cell Administration:

The eggs were wiped at the injection site with 70% ethanol and allowed to air dry. A hole measuring approximately 6mm was then drilled over the air cell in each egg using a "Dremel Moto-tool", model 270. The cutter employed deflected the shell fragments upwards and outwards. Remaining shell membrane fragments were removed with a small

forceps and the surface of the egg membrane visually examined for damage. The solvent or test sample was then deposited on the egg membrane with a model SB2 Syringe Microburet. Immediately following, the hole was sealed with ½" Scotch Brand transparent tape. Two additional groups of eggs were normally included with each air cell experiment; a group which had been drilled, shell membrane fragments removed, and sealed only and a group of control eggs which had received no treatments whatsoever.

2. Yolk Administration:

The eggs were placed within a Fisher Scientific "Isolator/Lab" equipped with plastic irises through which the hands and forearms were placed during injection. Prior to injection the eggs and miscellaneous required equipment were submitted to a fumigation of 1.8 grams of potassium permanganate crystals and 3.6 grams of 37% formaldehyde. The eggs were held in this atmosphere for 30 minutes prior to further handling.

Each egg was then wiped at the injection site with 70% ethanol and allowed to air dry. A small hole was engraved directly over the air cell with a Burgess model V-13 Vibro-Graver. Care was taken not to damage the membrane attached to the shell. The surface of the egg at the engraved site was vacuumed to remove the shell particles produced. The egg was then slid onto the needle of the Syringe Microburet with the egg horizontal on its long axis until the top of the egg reached the hub of the 1" - 25 ga. hypodermic needle. Following the injection of the material into the yolk sac, the egg was carefully withdrawn from the needle and the hole sealed with transparent tape. The hypodermic needle was carefully wiped with a sterile gauze pad prior to the next injection. As in the air cell administration, normally two additional groups of eggs were included in each yolk experiment; a group which had been drilled, pierced with the hypodermic needle and sealed only and a group of control eggs which had received no treatment other than fumigation.

Following the air cell and yolk injections the eggs were identified as to experiment and group with a No. 3 lead pencil and were then incubated as described above.

C. Test Profile:

The work was divided into one or more Preliminary Range Finding Experiments, two Dose-Response and Teratogenic Experiments, and Ancillary Investigations (Post Hatch Trials).

1. Preliminary Range Finding Experiments:

The objective of these trials was to locate the approximate LD-50 of the test sample. This data was used to design the dose levels for the Dose-Response trials. The test sample and solvent were administered by two routes; air cell and yolk, and at 0 and 96 hours of incubation. In general, at each route and time of incubation, 5 volumes of test sample dilution were administered together with 5 levels of solvent at the same volumes. Control eggs were also usually included as described above. Normally 10-20 eggs were used per group in these trials. When necessary these trials were repeated in an effort to locate the approximate LD-50 for the test compound.

Beginning on the 6th day of the incubation, the eggs set in the Preliminary Range-Finding Experiments were candled daily and non-viable embryos removed. These embryos were examined grossly for determination of developmental age and evidence of teratogenic effect, however, mortality was the main parameter in these trials. The remaining eggs were transferred to the hatching compartment on the 18th day of incubation and allowed to hatch. The resultant chicks and non-viable embryos were examined grossly for teratogenic effects and all pertinent data was recorded. An estimate of the LD-50 for the test compound was then made.

2. Dose-Response and Teratogenic Experiments:

Based upon information from the Preliminary Range-Finding Experiments, the Dose-Response Experiment was designed, employing 5 levels of sample dilution expected to produce mortality from the background level up through approximately 90%. Five volume levels of solvent were included as solvent controls at each route and time of administration. Normally 10 eggs/group were used in the solvent series with 50 eggs/group for the test sample dilutions. Twenty eggs/group were normally included for the drilled or pierced and non-treated controls. Two such experiments were conducted for each sample so that ultimately 100 eggs were tested on each test dilution at each route and stage of incubation.

The eggs set in these experiments were candled daily beginning on the 6th day of incubation and the non-viable embryos removed for examination as previously described. Where necessary, embryos were examined with the aid of a dissecting microscope. Remaining embryos were transferred to the hatching compartment on the 18th day of incubation and allowed to hatch. The apparently normal chicks were then removed from the hatching trays and examined externally for anomalies.

Remaining non-viable embryos and chicks which were alive but unable to hatch were individually examined externally for abnormalities. These non-viable embryos, chicks which were alive but unable to hatch, and a portion of the normal chicks were examined in one aspect by X-ray. The chicks and embryos which had been X-rayed and all remaining normal chicks were then examined internally for possible anomalies of the viscera. All pertinent data were recorded.

3. Post Hatch Trials:

Apparently normal chicks were chosen from one 50 egg experiment for this portion of the study.

Generally 20 chicks (straight-run) were wing banded from each level chosen and were placed in Jamesway electrically heated battery brooders. Central Soya Chick Starter was fed as the sole ration to 8 weeks of age and Central Soya Grower from 8 weeks of age to termination. These diets were non-medicated. The chicks chosen were usually from the approximate LD-50 and no-effect levels for the test compound from each route of administration and time of incubation. Negative control, untreated chicks, were also included. In some cases chicks were chosen from groups where a relatively high incidence of anomalies were seen rather than from the LD-50 or no-effect levels specifically. Body weight data were collected weekly through 4 weeks of age and bi-weekly to termination. Average group feed consumption was recorded periodically.

4. Histopathology:

A random sampling of birds from selected groups were specified for histologic examination. These chicks comprised 5 males and 5 females from the test groups selected and 5 males and 5 females from a negative control group. Groups to be sampled were selected on the basis of observations of specific effects and a judgment made as to what groups would give the most information from the limited histopathologic examination.

The chicks sacrificed were either day old or varying ages in a Post Hatch Trial. The following tissues were collected, trimmed, dehydrated, embedded in paraffin, sectioned and stained with hematoxilin and eosin:

1. Thyroid
2. Liver
3. Spleen
4. Pancreas
5. Lung
6. Heart
7. Kidney
8. Gonad
9. Bursa

The prepared slides were examined and remarkable alterations noted.

Discussion

1. The range finding experiments gave the following results:

Table 1
Mortality/Number Of Eggs

Dose Level Mg/Kg Sodium Nitrite	AC/0	AC/96	Y/0	Y/96
440.0	20/20	20/20		
366.7 (1)	17/20	20/20	20/20	20/20
220.0 (1)	13/40	36/40	19/20	8/20
110.0 (1)	5/40	22/40	16/20	2/20
73.3	6/20	11/20		
36.7 (1)	2/40	12/40	8/20	1/20
14.7 (1)	2/20	5/20	4/20	2/20

(1) Dose levels utilized in the dose response/teratogenic experiments

Computed (1) LD-50	Mg/Kg	Upper & Lower Limit:
AC/0	222.6	223 - 222
AC/96	94.0	95 - 93
Y/0	96	96 - 96
Y/96	237	237 - 237

(1) Statistical evaluation utilizing all birds from range finding and dose response studies.

2. Dose Response and Teratogenic experiment results for sodium nitrite are summarized in Tables 2, 3, 4, and 5. The mortality picture was similar to that observed in the range finding study.

Examining the incidence of abnormalities in terms of percent of eggs set, it will be noted that groups receiving solvent alone had an incidence of 7 to 15%, groups drilled and pierced had a 2.5 to 7.7% incidence, and the negative control groups had 6.4% incidence. The test groups showed a definite increase in the incidence of abnormalities. This was apparent for all routes and times of administration and was most evident with O/AC administration where groups receiving 366.7 and 220.0 mg/kg sodium nitrite contained 52 and 37% abnormal birds respectively.

In general for all routes and times, abnormalities were found in a wide variety of areas and structures but the most remarkable were dwarfism, head abnormalities involving beak, mandible and eyes, and visceral abnormalities where missing kidneys were most notable. 110 mg/kg sodium nitrite is apparently an effect level for O/AC, O/Y, and 96/AC while 96/Y does not show a response below 220 mg/kg. The groups receiving sodium nitrite 36.7 mg/kg 96/AC have an increased incidence of dwarfism and head abnormalities which indicate this is an effect level for this condition of administration.

Dwarfism was a frequent observation in the experiment on sodium nitrite. To clarify our designation of dwarfism we will explain our classification procedure. At the first

candling, day 5 or 6, we did not classify any embryos as retarded unless they were alive and definitely younger in development than 5 or 6 days. In subsequent candling any dead embryo judged by size to be 3 days behind in development was labeled as slight dwarfism, 4 days behind was labeled moderate dwarfism and 5 days or more behind was labeled severe dwarfism. If embryos were removed alive, 1 day behind was labeled slight, 2 days behind was labeled moderate and 3 days behind was labeled severe dwarfism. At hatch time an 18 day embryo was classified slightly dwarfed, a 17 day embryo was classified as moderate dwarfism and a 16 day embryo was classified as severe dwarfism. One might suspect that ,at the toxic levels administered, embryo development could be delayed due to metabolic or nutritional alterations that produced temporary growth depression which would not result in a permanent growth defect.

There were a variety of abnormalities of the head and even those which were represented by a single incidence in any one group carry a significance as a part of the high total abnormalities of the head in the test groups. Head abnormalities which were not observed in the controls for this experiment and have a very low flock background incidence deserve a special note. These are crossed beak, parrot beak, short maxilla, short beak, anophthalmia, microphthalmia, exophthalmia, cornea protruding, microblepharia, exencephaly, malformed skull, and acrania.

Visceral abnormalities although not great in number were remarkable for this type observed, the absence of such observations in the controls for this study, and the absence or low incidence of the conditions in the flock background data. Most notable observations were kidneys missing in 6 birds, 3 receiving 220 mg/kg sodium nitrite 0/AC and 3 receiving 110 mg/kg sodium nitrite 96/AC. Only two similar conditions were observed in the 3315 control eggs examined from this flock.

The X-ray examination did not reveal specific abnormalities not already noted on gross examinations.

3. Post Hatch trial included male and females from groups receiving 220 and 36.7 mg/kg AC/0, 36.7 and 14.7 mg/kg Y/0, 220 and 14.7 mg/kg AC/96, and 220 and 36.7 mg/kg Y/96, and were grown out to 14 weeks of age. In general, all groups had normal growth, feed consumption and development. No abnormalities related to grow out period were noted. Body weight data are summarized in Tables 6 and 7.

Since dwarfism was a frequent observation, it is important to determine whether this is a permanent growth effect resulting in an undersized adult or a temporary slowing of development where, in time, the adult will reach a normal size. In the limited post hatch growth study, no permanent dwarfing was noted.

4. Histopathology examinations were made on tissues from birds in the negative control (10 birds), 220 mg/kg AC/0 (9 birds), and 220 mg/kg Y/96 (11 birds) - groups that had been carried to 14 weeks of age. These data are summarized in Table 8, on Table 8, [redacted].

The histopathology tissue alterations observed in the control and test animals were minimal in nature, non specific, and randomly distributed between control and test animals.

Conclusion

The results of this study strongly suggest teratogenic activity of sodium nitrite on egg embryos when administered via yolk or air cell at 0 and 96 hour incubations. Air cell administration produced the most marked effect. Additional work should be done to confirm these results in other species.

Signed R M Bodden

By and for WARF Institute, Inc.

Date: July 31, 1974

Test Sample: Sodium Nitrite

Identification: FDA 71-9

Solvent System: 10% Absolute ethanol in sterile distilled H_2O

Breeder Flock: N-1

Preliminary Range Finding Experiments

Experiment No.	Initiated
5	11-12-71
8	12-16-72
20	3-23-72

Dose Response Experiments

Experiment No.	Initiated
25	4-24-72
36	7-10-72

Table 2
Sodium Nitrite
Dose Response Studies - Summary of Anomalies
Air Cell - 0 Hr

Dose MG/KG	366.7	220.0	110.0	36.7	14.7	Control 00.0 (1)	D (2)	C/C (3)	Flock (4) Backgroun
Eggs Set	100	100	100	100	100	100	40	140	
Deaths (5)	94	63	17	5	8	15	12	14	
75% Death Day (6)	5	11	17	6	16	17	19	20	
Abnormal Birds	51	37	15	7	5	11	3	9	
Functional									
weak						1			.06
Structural									
dwarfism	27	23	8	1	1	4	1	6	3.41
hair down								1	.21
clubbed down		1							.03
sparse down				1					.03
twins	1								---
Head									
parrot beak		1	1				1		.06
flexed mandible	2	5				1			.09
crossed beak		1	1						.09
short beak	1								---
short mandible	2	2					1		.09
anophthalmia		1	1						---
microphthalmia	22		1						.06
cornea protruding	1								---
microblepharia			1						.03
cleft palate								1	.03
exencephaly		1	1						.09
Skeletal									
Visceral									
celosomia			2						.06
missing kidney(s)		3							.06
small kidney(s)		1							.03
ovaries and testes present			1						---
small liver lobe		1							---
missing liver lobe		1							---
Limb									
perosis		3	3	3	2	2			.66
Toxic Response									
bloody yolk			1	2	1		1	1	.39
light down		1			1				.06
poorly healed navel						3			.33
hemorrhaged kidneys						1			.03
edema of head						1			.03

(1) Solvent control eggs at this injection time and route
(2) Drilled control eggs at this injection time
(3) Control/control eggs
(4) % of drilled, pierced, and control/control eggs from all 50 egg studies at WARF Institute using Flock N_1
(5) Deaths, infertile eggs, plus accidental deaths
(6) Day of incubation by which 75% of deaths (5) has occurred

Table 3
Sodium Nitrite
Dose Response Studies - Summary of Anomalies
Yolk - 0 Hr

Dose MG/KG	366.7	220.0	110.0	36.7	14.7	Control 00.0 (1)	P (2)	C/C (3)	Flock (4) Background
Eggs Set	100	100	100	100	100	100	40	140	
Deaths (5)	100	97	74	39	24	37	11	14	
75% Death Day (6)	2	3	5	5	5	4	5	20	
Abnormal Birds	18	30	29	9	4	15	--	9	
Functional									
Structural									
dwarfism	17	27	20	8	3	11		6	3.41
hair down								1	.21
sparse down		1							.03
clubbed down			2						.03
Head									
parrot beak		1							.06
crossed beak		2	1						.09
flexed mandible		2				1			.06
short mandible	1		1						.09
short maxilla		1							---
microphthalmia	1	2							.06
anophthalmia		1	2						---
eyelid dysplasia			1						.06
exencephaly		2		1					.09
cleft palate								1	.03
malformed skull	1								---
Skeletal									
spine malformed			1						---
Visceral									
celosomia			1	1					.06
small heart			1						---
small liver			1						---
dark testis				1					.03
enlarged testis				1					---
Limb									
perosis			1			2			.66
leg micromelia			1						---
tibias curved			1						---
metatarses curved			1						---
leg extended						1			.03
Toxic Response									
hemorrhaged body			1						---
hemorrhage near heart			1						---
poorly healed navel					1	2			.33
cord around neck			1						.06
bloody yolk								1	.39

(1) Solvent control eggs at this injection time and route
(2) Pierced control eggs at this injection time
(3) Control/control eggs
(4) % of drilled, pierced, and control/control eggs from all 50 egg studies at WARF Institute usi
Flock N_1
(5) Deaths, infertile eggs, plus accidental deaths
(6) Day of incubation by which 75% of deaths (5) had occurred

Table 4
Sodium Nitrite
Dose Response Studies - Summary of Anomalies
Air Cell - 96 Hr

Dose MG/KG	366.7	220.0	110.0	36.7	14.7	Control 00.0 (1)	D (2)	C/C (3)	Flock Backgroun (4)
Eggs Set	100	100	100	100	100	100	40	140	
Deaths (5)	100	67	61	22	8	7	2	14	
75% Death Day (6)	4	4	4	18	19	20	21	20	
Abnormal Birds	---	4	17	13	6	9	2	9	
Functional									
weak			1		1	2			.06
opisthotonos				1					---
Structural									
dwarfism		2	5	6	2	1		6	3.41
clubbed down				1	2				.03
dipygus		1							---
twins						1			---
hair down								1	.21
Head									
parrot beak				1					.06
crossed beak				1					.09
long mandible				1					---
exophthalmia			1						---
eyelid dysplasia			2						.06
ablepharia				1		1			---
acrania				1					.03
exencephaly				2					.09
cleft palate								1	.03
Skeletal									
spine duplication		1							---
Visceral									
enlarged heart			1						---
heart petechiae			1						---
omentum petechiae			1						---
missing kidney(s)			3						.06
small kidneys(s)			1			1			.03
small liver lobe(s)						2			---
enlarged liver lobe(s)						1			---
celosomia				1					.06
Limb									
perosis		2	6	1	1	4	2		.66
Toxic Response									
bloody yolk						1		1	.39
lung hemorrhage			1						---
eye hemorrhage			1						.03
edema around heart			1						---

(1) Solvent control eggs at this injection time and route
(2) Drilled control eggs at this injection time
(3) Control/control eggs
(4) % of drilled, pierced, and control/control eggs from all 50 egg studies at WARF Institute using Flock N
(5) Deaths, infertile eggs, plus accidental deaths
(6) Day of incubation by which 75% of deaths had occurred

Table 5
Sodium Nitrite
Dose Response Studies - Summary of Anomalies
Yolk - 96 Hr

Dose MG/KG	366.7	220.0	110.0	36.7	14.7	Control 00.0 (1)	(2) P	(3) C/C	Flock (4) Backgroun
Eggs Set	100	100	100	100	100	100	40	140	
Deaths (5)	100	22	9	9	5	7	8	14	
75% Death Day (6)	6	10	7	15	17	21	20	20	
Abnormal Birds	15	35	5	7	5	7	1	9	
Functional									
Structural									
dwarfism	12	5		3	2	2	1	6	3.41
clubbed down		2							.03
hair down		1						1	.21
sparse down		3							.03
Head									
crossed beak	1								.09
flexed mandible	2	1							.06
short mandible	1								.09
cleft palate								1	.03
Skeletal									
Visceral									
celosomia		1		1					.06
enlarged kidney(s)		1							.03
small kidney(s)						1			.03
small heart						1			---
Limb									
perosis		19	2	3	2	1			.66
curled toe(s)		1							---
leg micromelia		1							---
curved leg(s)		1							---
curved tibial(s)		1							---
crests on metatarses		1							---
Toxic Response									
bloody yolk			1			1		1	.39
kidney hemorrhage					1				---
poorly healed navel		4	1			2			.33
achromia			1						---

(1) Solvent control eggs at this injection time and route
(2) Pierced control eggs at this injection time
(3) Control/control eggs
(4) % of drilled, pierced, and control/control eggs from all 50 egg studies at WARF Institute using Flock N
(5) Deaths, infertile eggs, plus accidental deaths
(6) Day of incubation by which 75% of deaths (5) had occurred

Table 6 Body Weight Data - Post Hatch Response

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(Males)

Test Dose (1)	Time/Route	Average Individual Body Weight - Grams								
		Week 1	2	3	4	6	8	10	12	14
---	Control/Control	83	161	239	324	577	832	1122	1315	1576
220.0	0/AC	72	126	201	280	482	727	1033	1185	1443
36.7	0/AC	75	143	228	309	565	816	1159	1378	1653
36.7	0/Y	71	155	230	301	544	796	1127	1366	1595
14.7	0/Y	70	151	229	324	547	842	1150	1380	1641
220.0	96/AC	75	140	224	321	585	858	1210	1485	1754
14.7	96/AC	69	139	210	305	548	833	1157	1355	1504
220.0	96/Y	73	139	206	298	508	730	1052	1207	1561
36.7	96/Y	67	151	227	324	897	907	1147	1335	1576

(1) Milligrams/Kilogram of body weight.

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Table 7 Body Weight Data - Post Hatch Response

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(Females)

Test Dose (1)	Time/Route	Average Individual Body Weight - Grams								
		Week 1	2	3	4	6	8	10	12	14
---	Control/Control	78	149	214	292	490	708	932	1088	1267
220.0	O/AC	67	124	189	262	455	689	920	1067	1214
36.7	O/AC	74	138	201	272	471	619	839	947	1100
36.7	O/Y	74	148	215	283	497	723	945	1110	1293
14.7	O/Y	69	136	208	291	486	681	890	1030	1224
220.0	96/AC	77	143	212	295	497	721	1019	1115	1287
14.7	96/AC	70	137	202	287	484	676	903	1035	1230
220.0	96/Y	68	125	178	250	414	670	894	983	1171
36.7	96/Y	79	148	212	304	494	718	973	1141	1306

(1) Milligrams/Kilogram of body weight.

Table 8
Sodium Nitrite
Histopathology - Grow-Out Birds

Histologic Observations	Negative Control (10)	0/AC 220 MG/KG (9)	96/Y 220 MG/KG
Thyroid			
focal areas of cellular infiltration	1		2
area of cellular infiltration under capsule	1		
peripheral area of cellular infiltration			1
Lungs			
congested	3		
Heart			
focal areas of cellular infiltration	1		
Spleen			
peripheral pigmentation	8	4	2
diffuse pigmentation		5	9
Liver			
diffuse pigmentation	1	2	1
peripheral pigmentation	6	2	1
focal areas of cellular infiltration	9	6	9
Proventriculus			
focal areas of cellular infiltration	1		
Pancreas			
degenerate	1		
Kidney			
focal area of cellular infiltration		1	6
Gonads			
testicles immature	1	3	5
testicles degenerate	1	1	1
focal areas of cellular infiltration			1
Bone Marrow			
numerous fat vacuoles	2		

Table 8
Sodium Nitrite
Gross Pathology - Grow-Out Birds

Gross Observations	Negative Contol (10)	O/AC 220 MG/KG (9)	96/Y 220 MG/K
Kidneys			
no kidney			1
enlarged kidney			1
hemorrhage	1	1	
mottled		1	

MADISON, WISCONSIN

Sodium Nitrite

Errors In FDA Computer Print-Outs

Range Finding or Dose Response	Page	Incubation Date	Group	Error
DR	574	4/24/72	306	Bird 5 is coded for bei dwarfed twice
DR	589	7/10/72	334	Bird 2 is too young to coded as upside down - disregard
DR	607	4/24/72	345	Bird 3 is too young to be coded as upside down

These codings were not included in our summary tables for the final report but are included in the FDA print-out summaries.